Litton BIONETICS

MUTAGENICITY EVALUATION

G22

OF

CALCIUM SORBATE
00749Z-55-9
FDA 75-73

FINAL REPORT

GRM116

5516 Nicholson Lane
Kensington, Maryland
20795

Evaluation of Chemicals for Toxic & Teratogenic Effects Using the Chick Embryo As the Test System-FDA Contract #71-331 **Sodium Nitrate**: FDA 71-7
No Date

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SUBMITTED TO

FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION AND WELFARE
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LBI PROJECT NO. 2672

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Litton BIONETICS

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EVALUATION SUMMARY

The test compound, 00749Z559, did not exhibit mutagenic activity in any of the assays employed in these studies.

DATE: December 30, 1976

SPONSOR: U.S. Food and Drug Administration

SUBJECT: Evaluation of Test Compound Calcium Sorbate, 00749Z-55-9

I. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

II. MATERIALS

A. Test Compound

1. Date Received: October 21, 1976

2. Description: White powder

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

Yeast Strain: *Saccharomyces cerevisiae*, strain D4

Bacteria Strains: *Salmonella typhimurium*, strains TA-1535
TA-1537
TA-1538
TA-98
TA-100

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

Component	Final Concentration/ml
1. TPN (sodium salt)	4 µmoles
2. Glucose-6-phosphate	5 µmoles
3. Sodium phosphate (dibasic)	100 µmoles
4. $MgCl_2$	8 µmoles
5. KCl	33 µmoles
6. Homogenate fraction equivalent to 25 mg of wet tissue.	

D. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from tissues of the following mammalian species: Mouse - ICR random bred adult males; rat - Sprague-Dawley adult males; and monkey - Macaca mulatta adult males.

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1

POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

Assay	Chemical[a]	Solvent	Probable Mutagenic Specificity
Nonactivation	Methylnitrosoguanidine	Water or saline	BPS[b]
	Ethylmethanesulfonate	Water or saline	BPS[b]
	2-Nitrofluorene	Dimethylsulfoxide[c]	FS[b]
	Quinacrine mustard	Water or saline	FS[b]
Activation	Dimethylnitrosamine	Water or saline	BPS[b]
	2-Acetylaminofluorene	Dimethylsulfoxide[c]	FS[b]
	8-Aminoquinoline	Dimethylsulfoxide[c]	FS[b]
	2-Aminoanthracene	Dimethylsulfoxide[c]	BPS[b]

[a] Concentrations given in the Results Section
[b] BPS = base-pair substitution; FS = frameshift
[c] Previously shown to be non-mutagenic

III. METHODS

A. Toxicity

The solubility, toxicity and doses for the test chemical were determined prior to screening.

The test chemical was tested for toxicity against specific indicator strains over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival concentrations and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for the chemical with a given strain, then a maximum dose of 5% (w/v) was used.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.

B. Plate Tests (Overlay Method)

Approximately 10^8 cells from an overnight culture of each indicator strain were added to test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For nonactivation tests, the three dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests 0.5 ml of a 9,000 x g tissue supernatant and required cofactors (core reaction mixture) were added to the overlay tubes. Three dose levels of the test chemical were added to the appropriate tubes, which were then mixed and the contents poured over the surface of a minimal agar (selective medium) plate and allowed to solidify. The plates were incubated for 48 to 72 hours at 37°C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using positive compounds that are active directly and those that require metabolic activation were run with each assay.

C. Suspension Tests

1. Nonactivation

Bacteria and yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of 1×10^{10} cells/ml and 5×10^9 cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in plastic, 24-well tissue culture plates (Linbro). Cells plus appropriate volume(s) of the test chemical were added to the wells to give a final volume of 1.5 ml. The solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the plates were set on ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium in reversion experiments. Samples from a 10^{-1} dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

2. Activation

Bacteria and yeast cells were grown and prepared as described in the nonactivation tests. Measured amounts of the test and control chemicals plus 0.25 ml of the stock-cell suspension were added to wells of the Linbro plate containing the appropriate tissue fraction and reaction mixture. All flasks (bacteria and yeast) were incubated at 37°C with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for nonactivation tests.

D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (except monkeys) sufficient to provide the necessary quantities of tissues were killed by cranial blow, decapitated and bled. Monkey tissues were obtained from freshly killed and bled male rhesus monkeys. Organs were immediately dissected from the animals using aseptic techniques and placed in ice-cold 0.15M KCl. Upon collection of the desired quantity of organs, they were washed twice with fresh KCl and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies. Protein and P-448 determinations were made for each lot of homogenate.

E. Data Recording and Reporting

1. Plate test assays

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were entered into a computer program designed to print out all data by test. The data are presented as revertants per plate for each indicator strain employed in the assay. The positive and solvent controls are provided as reference points.

2. Suspension assays

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, on data processing forms. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. Other relevant experimental data were recorded on experimental definition forms. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated. The data were then processed and printed from a computer program. All raw data sheets are dated and signed by the responsible technician.

IV. RESULTS SECTION

A. Solubility Properties of the Test Compound

1. Name or code designation of the test compound: 00749Z-55-9

2. Test solvent: DMF

3. Solubility of the test compound under treatment conditions:
Soluble under test conditons.

4. Additional comments: White powder

B. Toxicity and Dosage Determinations for the Test Compound

1. Test date for toxicity determination:

2. The 50% survival level was determined for bacteria and yeast indicator organisms by conducting survival curves with the test compound at the following concentrations:

Percent Concentration (w/v or v/v)

5.0
0.5
0.05
0.005
0.0005

3. Concentrations of the test compound used in the mutagenicity tests:

	Percent Concentration	
Test Doses	Bacteria	Yeast
1/4 50% Survival	0.235	0.4
1/2 50% Survival	0.470	0.8
50% Survival	0.940	1.6

C. Plate Test Results

The plate test results are summarized in the following table. The values presented in this table are the number of revertants per plate.

D. Suspension Assay Results

The suspension test results for the test compound are summarized in the tables following the plate test summary. The values presented in these tables are the calculated mutation frequencies for each control and experimental test point. The first table of the suspension set presents the results for the nonactivation assays, and the second table through the fourth table of the suspension set presents the results for the activation assays. A listing of computer codes and abbreviations is included for reference. Tabulation of all raw data is provided in the Appendix.

SUMMARY OF TEST RESULTS

PLATE TESTS

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: 007492559

B. TEST DATE: NOV. 30, 1976

TEST		SPECIES	TISSUE	REVERTANTS PER PLATE									
				TA-1535		TA-1537		TA-1538		TA-98		TA-100	
				1	2	1	2	1	2	1	2	1	2
1. NON-ACTIVATION													
SOLVENT CONTROL*		---	---	19	26	12	22	28	17	58	43	145	110
POSITIVE CONTROL**		---	---	>1000	>1000	>1000	460	>1000	>1000	>1000	>1000	>1000	>1000
TEST	0.94000 %	---	---	16	16	20	23	41	23	44	89	150	130
	0.47000 %	---	---	17	20	32	49	35	30	46	48	119	121
	0.23500 %	---	---	17	15	19	22	25	24	50	72	103	147
2. ACTIVATION													
SOLVENT CONTROL*		MOUSE	LIVER	17	18	30	33	27	25	57	67	154	128
		RAT	LIVER	35	16	22	20	20	13	72	58	215	206
		MONKEY	LIVER	32	32	23	35	41	36	88	107	221	267
POSITIVE CONTROL***		MOUSE	LIVER	111	110	210	187	>1000	>1000	>1000	>1000	>1000	>1000
		RAT	LIVER	59	65	685	675	289	276	617	631	688	658
		MONKEY	LIVER	107	198	634	467	430	302	623	452	>1000	>1000
TEST	0.94000 %	MOUSE	LIVER	17	12	17	11	10	6	38	39	46	47
	0.47000 %	MOUSE	LIVER	9	14	31	34	14	19	47	45	94	130
	0.23500 %	MOUSE	LIVER	12	12	43	27	10	13	59	59	146	139
	0.94000 %	RAT	LIVER	14	19	29	26	15	22	21	30	57	69
	0.47000 %	RAT	LIVER	21	25	46	44	15	11	37	41	162	178
	0.23500 %	RAT	LIVER	28	20	40	46	14	4	53	54	128	180
	0.94000 %	MONKEY	LIVER	20	16	8	13	11	15	33	50	111	141
	0.47000 %	MONKEY	LIVER	21	26	30	39	15	17	48	41	191	115
	0.23500 %	MONKEY	LIVER	40	28	45	44	16	9	86	73	235	240

* NON-ACTIVATION ASSAYS CONSIST OF THE CELLS PLUS THE TEST COMPOUND VEHICLE (SOLVENT). FOR ACTIVATION ASSAYS, THE OVERLAY CONTAINS THE ACTIVATION SYSTEM PLUS THE TEST COMPOUND VEHICLE.

** TA-1535 MNNG 2 UG/PLATE
TA-1537 QM 20 UG/PLATE
TA-1538 NF 100 UG/PLATE
TA-98 NF 100 UG/PLATE
TA-100 MNNG 2 UG/PLATE

*** TA-1535 ANTH 100 UG/PLATE
TA-1537 AMQ 100 UG/PLATE
TA-1538 AAF 100 UG/PLATE
TA-98 AAF 100 UG/PLATE
TA-100 ANTH 100 UG/PLATE

NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS(UL) OR MICROGRAMS(UG) PER PLATE.

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 02/24/77

NONACTIVATION COMPOUND 007492559

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
NAN		93.00	10.00	24.95	10.10	5.39	6.84	8.58	CONTROLS
NAP		891.53	481.64	178.71	195.22	143.51	249.25	212.69	
NA1		17.46	2.72	16.57	7.90	2.70	7.12	7.79	TEST DATA
NA2		38.89	3.32	14.20	10.62	3.38	9.51	8.65	
NA3		35.42	4.51	27.71	10.54	2.75	7.30	7.11	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 02/24/77

SPECIES ICRFLO/MOUSE COMPOUND 00749Z559

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	43.20	4.97	4.23	12.17	3.58	9.55	4.36	NEGATIVE CONTROLS
ACT	A-C	32.56	5.02	2.38	7.39	2.69	9.50	1.67	
ACT	ALI	31.93	4.39	3.28	10.83	8.14	20.64	8.85	
ACT	ALU	44.37	4.92	5.65	11.66	5.45	6.36	1.54	
ACT	PLI	37.00	119.24	67.15	133.72	64.02	60.83	54.32	POSITIVE CONTROLS
ACT	PLU	27.32	5.17	1.83	42.72	9.73	8.81	4.02	
ACT	LII	27.93	6.39	1.73	12.68	8.88	10.73	3.14	TEST COMPOUND
ACT	LI2	39.38	7.42	2.98	4.23	4.12	10.55	8.11	
ACT	LI3	40.99	3.60	2.01	17.20	5.47	11.66	5.55	
ACT	LUI	37.68	4.88	2.08	14.55	2.13	5.69	5.38	
ACT	LU2	35.86	6.64	2.15	9.84	2.91	12.18	6.99	
ACT	LU3	32.30	5.05	1.69	8.90	3.40	8.64	5.98	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 02/24/77

SPECIES SPRDAW/RAT COMPOUND 007492559

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	45.96	6.79	10.81	23.31	3.75	6.88	5.37	NEGATIVE CONTROLS
ACT	A-C	49.21	8.71	3.14	10.64	3.67	6.33	4.13	
ACT	ALI	49.80	6.91	3.60	6.37	10.48	12.69	5.55	
ACT	ALU	52.54	6.93	6.99	6.71	6.43	8.77	4.94	
ACT	PLI	46.44	85.50	44.74	23.28	109.88	74.65	64.30	POSITIVE CONTROLS
ACT	PLU	38.92	6.38	5.64	566.01	86.28	8.88	3.84	
ACT	LII	34.05	6.19	6.18	8.00	5.69	8.23	5.84	TEST COMPOUND
ACT	LI2	43.60	5.92	2.46	12.23	9.48	7.98	5.35	
ACT	LI3	57.72	4.62	2.92	7.87	6.02	8.93	4.43	
ACT	LUI	47.48	8.21	4.49	5.40	2.12	8.28	4.17	
ACT	LU2	43.86	4.76	1.63	15.27	2.64	6.58	4.12	
ACT	LU3	45.95	6.79	2.92	10.08	3.99	6.47	4.92	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 02/24/77

SPECIES RHESUS/MONKEY COMPOUND 007492559

TEST	ORG	TA100 HIS EX-8	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	51.32	123.00	9.02	2.18	9.33	3.68	6.24	4.08	NEGATIVE CONTROLS
ACT	A-C	32.77	85.82	2.67	8.66	7.57	5.62	4.91	4.70	
ACT	ALI	45.20	91.19	2.17	3.79	7.46	13.68	7.60	8.01	
ACT	ALU	45.52	104.96	3.46	4.18	8.33	7.27	6.58	5.25	
ACT	PLI	39.48	218.23	117.77	72.02	224.19	100.30	51.53	50.43	POSITIVE CONTROLS
ACT	PLU	41.41	79.80	45.13	3.61	10.40	8.69	6.23	4.20	
ACT	LII	79.65	4.35	3.35	3.57	8.35	6.08	6.49	7.45	TEST COMPOUND
ACT	LI2	38.37	105.19	3.68	4.06	7.55	5.24	5.47	6.71	
ACT	LI3	44.52	123.88	2.33	2.58	9.18	7.04	5.96	5.88	
ACT	LUI	44.93	3.16	7.56	1.88	10.06	3.02	5.86	5.70	
ACT	LU2	65.67	74.81	2.15	1.62	7.37	5.42	4.31	3.87	
ACT	LU3	100.21	92.96	2.33	3.17	8.99	4.17	5.01	1.08	

DATA TABLE TERMS AND ABBREVIATIONS

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
COMPOUND	Client designated compound number appears in this column.
TEST CODES	NAN = Nonactivation: Solvent Control NAP = Nonactivation: Positive Control NA1 = Nonactivation: Test Compound Dose 1 NA2, etc. = Reflects the other dose level(s) A+C = Negative Chemical Control for ACP A-C = Activation: Solvent Control ALI or A+T = Activation: Homogenate Control (Liver) ALU = Activation: Homogenate Control (Lung) ACP = Activation: Positive Control ACT = Activation Test LI = Liver Tissue Activation Fraction LU = Lung Tissue Activation Fraction KI = Kidney Tissue Activation Fraction TE = Testes Tissue Activation Fraction 1,2, etc. = Dose Levels
CONCENTRATION	All test compound dose levels are expressed as a whole number followed by an exponent (negative) identified by the appropriate units. Example: 0025-2PCT = 0.25 percent concentration
POPU	Total number of viable cells in the plating sample raised to some exponent printed directly below the abbreviation (i.e., EP + 6 = x 10^6).
MUT 1	Total number of mutants or convertants obtained from the sample plated raised to some exponent printed directly below the abbreviation (i.e., EP + 0 = 10^0). For strain D4, MUT 1 represents the number of ADE+ convertants.
MUT 2	Only used for strain D4 and represents the number of TRY+ convertants in the plated sample.
FREQ 1	The calculated mutation or gene conversion frequency times the negative exponent written directly below. For strain D4, FREQ 1 represents the ADE+ value.
FREQ 2	Only used for strain D4 and represents the TRY+ conversion frequency.
CONTAM	Presence of contamination on any plates.

DATA TABLE TERMS AND ABBREVIATIONS (continued)

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
AAF	2-Acetylaminofluorene
DMSO	Dimethylsulfoxide
DMN	Dimethylnitrosamine
EMS	Ethylmethanesulfonate
QM	Quinacrine Mustard
NF	Nitrofluorene
ANTH	2-Amino Anthracene
AMQ	8-Amino Quinoline
SPECIES	Animal Strains
SPRDAW	Sprague Dawley Rats
ICRFLO	Flow ICR Random Bred Mice
RHESUS	Rhesus Monkey (Macaca mulatta)
MIXEDB	Dog, Mixed Breed
NEWZEA	New Zealand White Rabbit
UG	Microgram
UM	Micromole
ADE	Adenine
TRY	Tryptophan

V. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound, 007492559, was evaluated for genetic activity in a series of *in vitro* microbial assays with and without metabolic activation. The following results were obtained:

A. *Salmonella typhimurium*

1. Plate tests

The results of these tests were negative.

2. Nonactivation suspension tests

The results of these tests were negative.

3. Activation suspension tests

The results of these tests were negative.

B. *Saccharomyces cerevisiae*

1. Nonactivation suspension tests

The results of these tests were negative.

2. Activation suspension tests

The results of these tests were negative.

C. Conclusions

The test compound, 007492559, did not exhibit mutagenic activity in any of the assays employed in these studies.

Submitted by:

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Director
Department of Genetics

Reviewed by:

Robert J. Weir, Ph.D. — Date 3/24/77
Vice President

VI. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and cells are incubated in the overlay for 2-3 days, and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test.

- The small number of cell divisions permits potential mutagens to act on replicating DNA which is often more sensitive than non-replicating DNA.
- The combined incubation of the compound and the cells in the overlay permit constant exposure of the indicator cells for 2-3 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs dose levels that are selected such that the highest dose will show slight toxicity (as determined by subjective criteria) and several doses ranging down 1 to 2 logs lower.

B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. Factors which may modify dose response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced and the compound will not appear to be mutagenic.

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

1. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

2. Strains TA-98, TA-100, and D4

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

3. Pattern

Because TA-1535 and TA-100 were both derived from the same parental strain (G-46) and because TA-1538 and TA-98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a given strain, e.g. TA-1537, responds to a mutagen in nonactivation tests it will generally do so in activation tests. (The converse of this relationship is not expected.) While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

4. Reproducibility

If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data loses significance.

The preceding criteria are not absolute and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.

VII. EXPLANATION OF EVALUATION PROCEDURES FOR SUSPENSION ASSAYS

Data obtained from mutagenicity tests are evaluated on a test by test basis followed by an examination of the total response pattern using all the data. To facilitate this type of evaluation, we have prepared two separate formats in which data are processed. The first is the Compound Summary Backup Detail Sheet, which details the essential raw data from each experiment showing surviving population counts, total mutant or convertant counts, as well as, calculated mutation frequencies. This format permits close examination of each set of test data. The following considerations are part of any assessment.

A. Surviving Population Counts

A certain level of chemically-induced toxicity is anticipated, but occasionally isolated tests or groups of tests show very low (<25%) survival compared to the tissue controls. Such isolated decreases may result from improper dilution procedures or defective growth media and decrease confidence in the calculated mutation frequencies especially if the total mutant counts appear unaffected. Data of this type are generally unacceptable and these experiments are routinely repeated at a lower dose level to reduce killing and increase confidence in the nature of the response.

B. Total Mutant Counts

For nonmutagens, the mutant/surviving population ratio should be roughly equivalent for each test point in a given experiment. If the cell number drops in response to killing, the mutant number should decrease proportionately. A mutagenic chemical, however, will produce an altered mutant/surviving population ratio. Mutant numbers as well as calculated frequencies are compared to the negative control data. In certain instances, the mutant frequencies will increase with little or no change in the absolute number of mutants especially where the test chemical is toxic. Data of this type, although not necessarily aberrant, or even rare, must be viewed with special care to ensure that the increased frequencies were not the result of selective toxicity of the test chemical for the *his*$^-$ cells. This phenomenon, referred to as selection, can lead to erroneous conclusions. Thus we attempt to keep the surviving population of cells high and look for positive responses that show increases in both numbers of mutants and mutation frequencies. Again, occasional isolated fluctuations in mutant counts are found that can be attributed to improper pipetting or media contamination. These fluctuations are usually easy to identify by inspection of the other data points in the experiment which will be negative.

C. Dose Response Phenomena

Dose-related increases in mutants and mutation frequencies are the most convincing data to have in assessing mutagenic activity of chemicals. In some cases, however, dose-related increases are not observed for mutagens. This depends considerably on the dose levels selected. The figure on the following page illustrates how one might obtain various types of dose-related responses by a mutagen based solely on dose selection. It also emphasizes the need to keep dose levels within a relatively low range of toxicity so that data are consistently on the uphill side of the hypothetical curve.

D. Control Tests

Positive and negative control tests are conducted with each experiment and consist of direct acting positive agents for nonactivation assays and chemicals that require metabolic transformation for activation assays. In nonactivation assays, the NAN control contain the test chemical solvent plus cells, but no chemical, and is used as a reference to assess the level of response obtained in the various tests. It is not possible at this time to put precise cut-off points where negative responses become positive responses. A statistical component for our computer program is under development and will be included when available. Positive controls are only used as relative reference points and to demonstrate that the system is functioning with known mutagens. In activation assays, three types of negative controls are run: (1) A solvent control minus the chemical and minus the activation system (A-C); (2) a control plus the positive control chemical minus the activation system (A+C); and (3) a control containing the activation system and the test chemical solvent (ALI or ALU). All three controls are used collectively to assess the level of response in the various activation tests. A chemical may appear positive when compared to an A-C control but not when compared to an A+T control. The value of each of the above controls with respect to their weight in evaluation is ALI or ALU > A-C > A+C.

The other data format is the Compound Frequency Summary Report sheet in which all the calculated frequencies obtained for a given compound are displayed in a table. This format permits an overview of all data. The points form a matrix of information that should present a consistent pattern. Nonmutagens should produce a matrix with data frequencies clustered around the negative control values. Occasional random high or low fluctuations are not uncommon and seldom indicate true genetic activity. Mutagenic chemicals should, on the other hand, produce a set of consistent responses that demonstrate a logical pattern. The patterns depend on the mutagenic specificity of the chemical but can be easily recognized in the Compound Frequency Summary Report format.

These mutagenicity assays are designed to optimize the probability of recognizing mutagens from nonmutagens and, in most cases, they work well. Occasionally, the data points are such that a definitive conclusion cannot be made without additional data.

APPENDIX

Tabulation of Data

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 633401 CONTRACT 22376-2102 DETECTOR TA100 SPECIES PROJECT 02672 / DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	NAN		SOLVENT	0514	0478	93.00	0
	NAP		EMS 0.066%	0744	6633	891.53	0
007497559	NA1		0094-2 PCT.	1243	0217	17.46	0
007497559	NA2		0047-2 PCT.	0900	0350	38.89	0
007497559	NA3		0235-3 PCT.	1152	0408	35.42	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 633702 DETECTOR TA1535 SPECIES / DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	0480	0048	10.00	0
	NAP		EMS 0.2%	0828	3988	481.64	0
007492559	NA1		0094-2 PCT.	0515	0014	2.72	0
007492559	NA2		0047-2 PCT.	0602	0020	3.32	0
007492559	NA3		0235-3 PCT.	0732	0033	4.51	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 633406 DETECTOR TA1537 SPECIES / DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	1038	0259	24.95	0
	NAP		QM 13 UG/ML	0681	1217	178.71	0
007497559	NA1		0094-2 PCT.	1255	0208	16.57	0
007497559	NA2		0047-2 PCT.	1592	0226	14.20	0
007497559	NA3		0235-3 PCT.	1050	0291	27.71	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 633703 DETECTOR TA1538 SPECIES / DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	NAN		SOLVENT	0485	0049	10.10	0
	NAP		NF 667 UG/ML	0418	0816	195.22	0
007492559	NA1		0094-2 PCT.	0595	0047	7.90	0
007492559	NA2		0047-2 PCT.	0433	0046	10.62	0
007492559	NA3		0235-3 PCT.	0446	0047	10.54	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 634101 CONTRACT 22376-2102 DETECTOR TA98 SPECIES PROJECT 02672 / DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	NAN		SOLVENT	0501	0027	5.39	0
	NAP		NF 667 UG/ML	1255	1801	143.51	0
007492559	NA1		0094-2 PCT.	1076	0029	2.70	0
007492559	NA2		0047-2 PCT.	1362	0046	3.38	0
007492559	NA3		0235-3 PCT.	1526	0042	2.75	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 633405 DETECTOR 0000D4 SPECIES / DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	NAN		SOLVENT	1841	0126	0158	6.84	8.58	0
	NAP		EMS 1.0 %	0733	1827	1559	249.25	212.69	0
007497559	NA1		0016-1 PCT.	1811	0129	0141	7.12	7.79	0
007497559	NA2		0008-1 PCT.	1388	0132	0120	9.51	8.65	0
007497559	NA3		0004-1 PCT.	1590	0116	0113	7.30	7.11	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 633602 DETECTOR TA100 SPECIES ICRFLO/MOUSE DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	1301	0562	43.20	0
	A-C		SOLVENT	1508	0491	32.56	0
	ALI		TISSUE	1613	0515	31.93	0
	ALII		TISSUE	1564	0694	44.37	0
	ACP	LI	DMN 90 UM/ML	1411	0522	37.00	0
	ACP	LU	DMN 90 UM/ML	1768	0483	27.32	0
007497559	ACT	LII	0094-2 PCT.	0709	0198	27.93	0
007497559	ACT	LI2	0047-2 PCT.	1130	0445	39.38	0
007497559	ACT	LI3	0235-3 PCT.	1371	0562	40.99	0
007497559	ACT	LUI	0094-2 PCT.	0406	0153	37.68	0
007497559	ACT	LU2	0047-2 PCT.	1135	0407	35.86	0
007497559	ACT	LU3	0235-3 PCT.	1477	0477	32.30	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 634108 DETECTOR TA1535 SPECIES ICRFLO/MOUSE DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	0945	0047	4.97	0
	A-C		SOLVENT	1096	0055	5.02	0
	ALI		TISSUE	1049	0046	4.39	0
	ALU		TISSUE	1118	0055	4.92	0
	ACP	LI	DMN 90 UM/ML	0421	0502	119.24	0
	ACP	LU	DMN 90 UM/ML	0831	0043	5.17	0
007492559	ACT	LI1	0094-2 PCT.	0454	0029	6.39	0
007492559	ACT	LI2	0047-2 PCT.	0674	0050	7.42	0
007492559	ACT	LI3	0235-3 PCT.	1388	0050	3.60	0
007492559	ACT	LU1	0094-2 PCT.	0430	0021	4.88	2
007492559	ACT	LU2	0047-2 PCT.	0572	0038	6.64	0
007492559	ACT	LU3	0235-3 PCT.	1228	0062	5.05	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 634105 DETECTOR TA1537 SPECIES ICRFLO/MOUSE DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		AMQ 333 UG/ML	1535	0065	4.23	0
	A-C		SOLVENT	1346	0032	2.38	0
	ALI		TISSUE	0884	0029	3.28	0
	ALU		TISSUE	8372	0021	5.65	0
	ACP	LI	AMQ 333 UG/ML	0615	0413	67.15	0
	ACP	LU	AMQ 333 UG/ML	1808	0033	1.83	0
007497559	ACT	LI1	0094-2 PCT.	0866	0015	1.73	0
007497559	ACT	LI2	0047-2 PCT.	1142	0034	2.98	0
007497559	ACT	LI3	0235-3 PCT.	1343	0027	2.01	0
007497559	ACT	LU1	0094-2 PCT.	0578	0012	2.08	0
007497559	ACT	LU2	0047-2 PCT.	1022	0022	2.15	0
007497559	ACT	LU3	0235-3 PCT.	1246	0021	1.69	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 633502 DETECTOR TA1538 SPECIES ICRFLO/MOUSE DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0304	0037	12.17	0
	A-C		SOLVENT	0352	0026	7.39	0
	ALI		TISSUE	0434	0047	10.83	0
	ALU		TISSUE	0446	0052	11.66	0
	ACP	LI	ANTH 67 UG/ML	0433	0579	133.72	0
	ACP	LU	ANTH 67 UG/ML	0419	0179	42.72	0
007492559	ACT	LI1	0094-2 PCT.	0355	0045	12.68	0
007492559	ACT	LI2	0047-2 PCT.	0307	0013	4.23	0
007492559	ACT	LI3	0235-3 PCT.	0628	0108	17.20	0
007492559	ACT	LU1	0094-2 PCT.	0275	0040	14.55	0
007492559	ACT	LU2	0047-2 PCT.	0193	0019	9.84	0
007492559	ACT	LU3	0235-3 PCT.	0191	0017	8.90	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 634112 DETECTOR TA98 SPECIES ICRFLO/MOUSE DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+8	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	1845	0066	3.58	0
	A-C		SOLVENT	1671	0045	2.69	0
	ALI		TISSUE	0663	0054	8.14	0
	ALU		TISSUE	0734	0040	5.45	0
	ACP	LI	ANTH 67 UG/ML	0970	0621	64.02	0
	ACP	LU	ANTH 67 UG/ML	1387	0135	9.73	0
007492559	ACT	LI1	0094-2 PCT.	0338	0030	8.88	0
007492559	ACT	LI2	0047-2 PCT.	1237	0051	4.12	0
007492559	ACT	LI3	0235-3 PCT.	0878	0048	5.47	0
007492559	ACT	LU1	0094-2 PCT.	0751	0016	2.13	0
007492559	ACT	LU2	0047-2 PCT.	1237	0036	2.91	0
007492559	ACT	LU3	0235-3 PCT.	1176	0040	3.40	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 634901 DETECTOR 0000D4 SPECIES ICRFLO/MOUSE DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	1194	0114	0052	9.55	4.36	0
	A-C		SOLVENT	0600	0057	0010	9.50	1.67	0
	ALI		TISSUE	0407	0084	0036	20.64	8.85	0
	ALU		TISSUE	0912	0058	0014	6.36	1.54	0
	ACP	LI	DMN 90 UM/ML	1366	0831	0742	60.83	54.32	0
	ACP	LU	DMN 90 UM/ML	1044	0092	0042	8.81	4.02	0
007492559	ACT	LI1	0016-1 PCT.	0606	0065	0019	10.73	3.14	0
007492559	ACT	LI2	0008-1 PCT.	1356	0143	0110	10.55	8.11	0
007492559	ACT	LI3	0004-1 PCT.	1261	0147	0070	11.66	5.55	0
007492559	ACT	LU1	0016-1 PCT.	1301	0074	0070	5.69	5.38	0
007492559	ACT	LU2	0008-1 PCT.	1273	0155	0089	12.18	6.99	0
007492559	ACT	LU3	0004-1 PCT.	0752	0065	0045	8.64	5.98	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 633603 DETECTOR TA100 SPECIES SPRDAW/RAT DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	1249	0574	45.96	0
	A-C		SOLVENT	1016	0500	49.21	0
	ALI		TISSUE	1275	0635	49.80	0
	ALU		TISSUE	1203	0632	52.54	0
	ACP	LI	DMN 90 UM/ML	1111	0516	46.44	0
	ACP	LU	DMN 90 UM/ML	1349	0525	38.92	0
007492559	ACT	LI1	0094-2 PCT.	0749	0255	34.05	0
007492559	ACT	LI2	0047-2 PCT.	1188	0518	43.60	0
007492559	ACT	LI3	0235-3 PCT.	1043	0602	57.72	0
007492559	ACT	LU1	0094-2 PCT.	0575	0273	47.48	0
007492559	ACT	LU2	0047-2 PCT.	1361	0597	43.86	0
007492559	ACT	LU3	0235-3 PCT.	1160	0533	45.95	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 633601 DETECTOR TA1535 SPECIES SPRDAW/RAT DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	0928	0063	6.79	0
	A-C		SOLVENT	0758	0066	8.71	0
	ALI		TISSUE	1013	0070	6.91	0
	ALU		TISSUE	1024	0071	6.93	0
	ACP	LI	DMN 90 UM/ML	0855	0731	85.50	0
	ACP	LU	DMN 90 UM/ML	1160	0074	6.38	0
007497559	ACT	LI1	0094-2 PCT.	0404	0025	6.19	0
007497559	ACT	LI2	0047-2 PCT.	0879	0052	5.92	0
007497559	ACT	LI3	0235-3 PCT.	1233	0057	4.62	0
007497559	ACT	LU1	0094-2 PCT.	0207	0017	8.21	0
007497559	ACT	LU2	0047-2 PCT.	0966	0046	4.76	0
007497559	ACT	LU3	0235-3 PCT.	0839	0057	6.79	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 634203 DETECTOR TA1537 SPECIES SPRDAW/RAT DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AMQ 333 UG/ML	0675	0073	10.81	0
	A-C		SOLVENT	0986	0031	3.14	0
	ALI		TISSUE	0750	0027	3.60	0
	ALU		TISSUE	0372	0026	6.99	0
	ACP	LI	AMQ 333 UG/ML	0523	0234	44.74	0
	ACP	LU	AMQ 333 UG/ML	1187	0067	5.64	0
007497559	ACT	LI1	0094-2 PCT.	0502	0031	6.18	0
007497559	ACT	LI2	0047-2 PCT.	1018	0025	2.46	0
007497559	ACT	LI3	0235-3 PCT.	0926	0027	2.92	0
007497559	ACT	LU1	0094-2 PCT.	0468	0021	4.49	0
007497559	ACT	LU2	0047-2 PCT.	1044	0017	1.63	0
007497559	ACT	LU3	0235-3 PCT.	1062	0031	2.92	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 633801 CONTRACT 22376-2102 DETECTOR TA1538 PROJECT 02672 SPECIES SPRDAW/RAT DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0133	0031	23.31	0
	A-C		SOLVENT	0188	0020	10.64	0
	ALI		TISSUE	0267	0017	6.37	0
	ALU		TISSUE	0164	0011	6.71	0
	ACP	LI	ANTH 67 UG/ML	0232	0054	23.28	0
	ACP	LU	ANTH 67 UG/ML	0153	0866	566.01	0
007492559	ACT	LI1	0094-2 PCT.	0175	0014	8.00	0
007492559	ACT	LI2	0047-2 PCT.	0188	0023	12.23	0
007492559	ACT	LI3	0235-3 PCT.	0178	0014	7.87	0
007492559	ACT	LU1	0094-2 PCT.	0315	0017	5.40	0
007492559	ACT	LU2	0047-2 PCT.	0203	0031	15.27	0
007492559	ACT	LU3	0235-3 PCT.	0258	0026	10.08	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 634204 CONTRACT 22376-2102 DETECTOR TA98 SPECIES SPRDAW/RAT PROJECT 02672 DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	1972	0074	3.75	0
	A-C		SOLVENT	1309	0048	3.67	0
	ALI		TISSUE	0582	0061	10.48	0
	ALU		TISSUE	0886	0057	6.43	0
	ACP	LI	ANTH 67 UG/ML	1599	1757	109.88	0
	ACP	LU	ANTH 67 UG/ML	0678	0585	86.28	0
007492559	ACT	LI1	0094-2 PCT.	0633	0036	5.69	0
007492559	ACT	LI2	0047-2 PCT.	0496	0047	9.48	0
007492559	ACT	LI3	0235-3 PCT.	0964	0058	6.02	0
007492559	ACT	LU1	0094-2 PCT.	1462	0031	2.12	0
007492559	ACT	LU2	0047-2 PCT.	1138	0030	2.64	0
007492559	ACT	LU3	0235-3 PCT.	1228	0049	3.99	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 634903 DETECTOR 000004 SPECIES SPRDAW/RAT DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	1526	0105	0082	6.88	5.37	0
	A-C		SOLVENT	1548	0098	0064	6.33	4.13	0
	ALI		TISSUE	1009	0128	0056	12.69	5.55	0
	ALU		TISSUE	1357	0119	0067	8.77	4.94	0
	ACP	LI	DMN 90 UM/ML	1199	0895	0771	74.65	64.30	0
	ACP	LU	DMN 90 UM/ML	1407	0125	0054	8.88	3.84	0
007497559	ACT	LI1	0016-1 PCT.	1336	0110	0078	8.23	5.84	0
007497559	ACT	LI2	0008-1 PCT.	1141	0091	0061	7.98	5.35	0
007497559	ACT	LI3	0004-1 PCT.	1310	0117	0058	8.93	4.43	0
007497559	ACT	LU1	0016-1 PCT.	1510	0125	0063	8.28	4.17	0
007497559	ACT	LU2	0008-1 PCT.	1625	0107	0067	6.58	4.12	0
007497559	ACT	LU3	0004-1 PCT.	1546	0100	0076	6.47	4.92	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 633701 DETECTOR TA100 SPECIES RHESUS/MONKEY DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	1210	0621	51.32	0
	A-C		SOLVENT	1727	0566	32.77	0
	ALI		TISSUE	1522	0688	45.20	0
	ALU		TISSUE	1751	0797	45.52	0
	ACP	LI	DMN 90 UM/ML	1530	0604	39.48	0
	ACP	LU	DMN 90 UM/ML	1466	0607	41.41	0
007492559	ACT	LI1	0094-2 PCT.	0860	0685	79.65	0
007492559	ACT	LI2	0047-2 PCT.	1501	0576	38.37	0
007492559	ACT	LI3	0235-3 PCT.	1633	0727	44.52	0
007492559	ACT	LU1	0094-2 PCT.	0345	0155	44.93	0
007492559	ACT	LU2	0047-2 PCT.	0670	0440	65.67	0
007492559	ACT	LU3	0235-3 PCT.	0469	0470	100.21	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEN
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 703502 DETECTOR TA100 SPECIES RHESUS/MONKEY DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+8	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	0639	0786	123.00	0
	A-C		SOLVENT	0536	0460	85.82	0
	ALI		TISSUE	0965	0880	91.19	0
	ALU		TISSUE	0826	0867	104.96	0
	ACP	LI	DMN 90 UM/ML	1031	2250	218.23	0
	ACP	LU	DMN 90 UM/ML	1228	0980	79.80	0
007497559	ACT	LI1	0094-2 PCT.	0391	0017	4.35	0
007497559	ACT	LI2	0047-2 PCT.	0501	0527	105.19	0
007497559	ACT	LI3	0235-3 PCT.	0896	1110	123.88	0
007497559	ACT	LU1	0094-2 PCT.	0411	0013	3.16	0
007497559	ACT	LU2	0047-2 PCT.	0397	0297	74.81	0
007497559	ACT	LU3	0235-3 PCT.	0639	0594	92.96	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 634801 DETECTOR TA1535 SPECIES RHESUS/MONKEY DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0510	0046	9.02	0
	A-C		SOLVENT	1088	0029	2.67	0
	ALI		TISSUE	1289	0028	2.17	0
	ALU		TISSUE	0953	0033	3.46	0
	ACP	LI	DMN 90 UM/ML	0754	0888	117.77	0
	ACP	LU	DMN 90 UM/ML	0308	0139	45.13	0
007497559	ACT	LI1	0094-2 PCT.	0388	0013	3.35	0
007497559	ACT	LI2	0047-2 PCT.	0869	0032	3.68	0
007497559	ACT	LI3	0235-3 PCT.	1290	0030	2.33	0
007497559	ACT	LU1	0094-2 PCT.	0291	0022	7.56	0
007497559	ACT	LU2	0047-2 PCT.	0605	0013	2.15	0
007497559	ACT	LU3	0235-3 PCT.	1071	0025	2.33	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 634301 DETECTOR TA1537 SPECIES RHESUS/MONKEY DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AMQ 333 UG/ML	1787	0039	2.18	0
	A-C		SOLVENT	1294	0112	8.66	0
	ALI		TISSUE	0792	0030	3.79	0
	ALU		TISSUE	0814	0034	4.18	0
	ACP	LI	AMQ 333 UG/ML	0486	0350	72.02	0
	ACP	LU	AMQ 333 UG/ML	0858	0031	3.61	0
007497559	ACT	LI1	0094-2 PCT.	0952	0034	3.57	0
007497559	ACT	LI2	0047-2 PCT.	0837	0034	4.06	0
007497559	ACT	LI3	0235-3 PCT.	1201	0031	2.58	0
007497559	ACT	LU1	0094-2 PCT.	0584	0011	1.88	0
007497559	ACT	LU2	0047-2 PCT.	1176	0019	1.62	0
007497559	ACT	LU3	0235-3 PCT.	0977	0031	3.17	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 634201 CONTRACT 22376-2102 DETECTOR TA1538 PROJECT 02672 SPECIES RHESUS/MONKEY DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0697	0065	9.33	0
	A-C		SOLVENT	0700	0053	7.57	0
	ALI		TISSUE	0670	0050	7.46	0
	ALU		TISSUE	0648	0054	8.33	0
	ACP	LI	ANTH 67 UG/ML	0277	0621	224.19	0
	ACP	LU	ANTH 67 UG/ML	0577	0060	10.40	0
007492559	ACT	LI1	0094-2 PCT.	0611	0051	8.35	0
007492559	ACT	LI2	0047-2 PCT.	0636	0048	7.55	0
007492559	ACT	LI3	0235-3 PCT.	0621	0057	9.18	0
007492559	ACT	LU1	0094-2 PCT.	0477	0048	10.06	0
007492559	ACT	LU2	0047-2 PCT.	0624	0046	7.37	0
007492559	ACT	LU3	0235-3 PCT.	0601	0054	8.99	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 634202 DETECTOR TA98 SPECIES RHESUS/MONKEY DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	2094	0077	3.68	0
	A-C		SOLVENT	1656	0093	5.62	0
	ALI		TISSUE	0563	0077	13.68	0
	ALU		TISSUE	0949	0069	7.27	0
	ACP	LI	ANTH 67 UG/ML	1006	1009	100.30	0
	ACP	LU	ANTH 67 UG/ML	0875	0076	8.69	0
007497559	ACT	LI1	0094-2 PCT.	0806	0049	6.08	0
007497559	ACT	LI2	0047-2 PCT.	1069	0056	5.24	0
007497559	ACT	LI3	0235-3 PCT.	1051	0074	7.04	0
007497559	ACT	LU1	0094-2 PCT.	1324	0040	3.02	0
007497559	ACT	LU2	0047-2 PCT.	1218	0066	5.42	0
007497559	ACT	LU3	0235-3 PCT.	1847	0077	4.17	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 634904 DETECTOR 0000D4 SPECIES RHESUS/MONKEY DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	1299	0081	0053	6.24	4.08	0
	A-C		SOLVENT	1425	0070	0067	4.91	4.70	0
	ALI		TISSUE	1211	0092	0097	7.60	8.01	0
	ALU		TISSUE	1505	0099	0079	6.58	5.25	0
	ACP	LI	DMN 90 UM/ML	1733	0893	0874	51.53	50.43	0
	ACP	LU	DMN 90 UM/ML	1573	0098	0066	6.23	4.20	0
007492559	ACT	LI1	0016-1 PCT.	1141	0074	0085	6.49	7.45	0
007492559	ACT	LI2	0008-1 PCT.	1133	0062	0076	5.47	6.71	0
007492559	ACT	LI3	0004-1 PCT.	1157	0069	0068	5.96	5.88	0
007492559	ACT	LU1	0016-1 PCT.	1246	0073	0071	5.86	5.70	0
007492559	ACT	LU2	0008-1 PCT.	1345	0058	0052	4.31	3.87	0
007492559	ACT	LU3	0004-1 PCT.	1297	0065	0014	5.01	1.08	0